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Solvent in Protein Interfaces: Molecular Dynamics Approach

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Water molecules are present ubiquitously in living cells. However, solvent contribution to protein-protein interactions is often ignored in protein-protein interactions studies. Previous work has suggested the importance of wet spots (residues interacting only through one water molecule) in description of protein interactions. We use a molecular dynamics approach to analyze solvent in protein interfaces. Our results show that residence time of water molecules in wet spots sites is found to be significantly higher than of water molecules on protein surfaces. In terms of free energy these water molecules are heterogeneous. Nevertheless, their contribution to the free energy of complex formation significantly changes the energy function of the system suggesting that water should be considered in detailed protein interface description.

1 Introduction

Water plays an extremely important role in all biological processes. Water molecules have been shown to be structurally conserved in protein complexes and to contribute thermodynamically to protein complex formation, while their residence time and diffusion characteristics in protein interfaces are distinct from bulk and surface solvent [1]. Despite all, solvent is often ignored in the analysis of protein-protein interactions.

In our previous work we have developed SCOWLP, which, taking into account interfacial solvent, classifies all interfacial protein residues of the PDB into three classes based on their interacting properties: dry (direct interaction), dual (direct and water-mediated interactions), and wet spots (residues interacting only through one water molecule) [2]. This study aims to gain insights into dynamic and energetic properties of solvent in protein interfaces using MD approach.

2 Methods

10 ns of MD productive runs were carried out in AMBER 8.0 using isothermal isobaric periodic boundary conditions and TIP3P water model. Interfacial interactions were defined by the SCOWLP criteria [2]. If the interacting heavy atoms of each wet spot counterpart were closer than 3.6 Å to water molecules, the wet spot site was considered to be occupied. A surface water site was defined by the volume with 100% total occupancy around one of the protein polar groups outside the interface. A bulk water site was defined similarly in terms of total occupancy. Residence time distribution density, maximum residence time (Tmax) and total occupancy were defined as the frequency of consecutively occupied frames, maximum number of consecutively occupied frames and the total time when the site was occupied, respectively. For free energy calculations, the double decoupling method of free energy perturbation was used [3]. The coupling parameter λ was varied

from 0 to 1 and back with a 0.01 step. The system was equilibrated for 10 ps for each l value followed by a 10 ps productive MD run.

3 Results

3.1 Residence Time of Water Molecules in Wet Spot Sites

The analysis of wet spot sites from 17 protein-peptide and protein-protein complexes suggests that residence time density distribution for each site is described as: $F(t) = Ct^{-k}$, where C is a normalization constant, and $k > 0$ is the only distribution parameter. k and T_{\max} were compared for wet spots, surface and bulk water sites. It was shown that both parameters significantly differ (at the level of t-Test p-value=0.05) for different sites, indicating that water molecules in wet spot sites are more stable than in bulk solvent or in surface hydration sites (Figure 1). At the same time, in each wet spot site there are many occupancy events that have as short residence as in bulk or surface sites. That agrees with the model proposed by Makarov et al. Here, the correlation function for residence time in hydration sites is decomposed into the sum of fast and slow diffusion exponent components, which characterize bulk water motions and specific for hydration site events, respectively [10]. Other theoretical and experimental studies obtained similar residence time values for different water sites, which vary from 1-10 ps for bulk solvent to $10^2 - 10^3$ ps for protein hydration sites, cavities and cores [1]. T_{\max} and k are well correlated (adjusted correlation coefficient $r=0.81$ for $\ln(T_{\max})$ k), while there was no correlation between total occupancy of the sites and T_{\max} ($r < 0.3$) because these parameters are independent and describe different kinetic characteristics of the site. While T_{\max} is defined only by the energy barrier required for the molecule to leave the site, total occupancy is also dependent on the energy barrier of water transfer from bulk solvent to the site. The residence time analysis suggests that the potential barriers for wet spots sites are significantly higher than those for surface sites.

3.2 Free Energy of Water Molecules in Wet Spot Sites

To determine if water molecules contribute energetically favorably to complex formation we calculated their free energy. As a first step, free energy of removing a water molecule from bulk solvent was calculated. Electrostatic and van der Waals components were equal to 8.2 and -2.2 kcal/mol, respectively, which agrees well with the results obtained from similar calculations correlated with experimental data [3]. The second step consisted of the transfer of a water molecule from the wet spot site to vacuum. The difference of these two energy components makes up the total energy of a water molecule transfer from bulk solvent to the wet spot site. The obtained results for several water sites of the SH3 domain complex 1uj0 show that the sites are very heterogeneous. In particular, the free energy of water molecule transfer from the site formed by the carboxyl oxygen of Glu12 in the SH3 domain and the side-chain of Arg64 in the ligand is -1.4 kcal/mol, meaning a favorable impact of a water molecule on the complex formation. The calculations carried out for another site formed by the side-chain of Asp34 in the SH3 domain and the side-chain of Asn66 in the ligand revealed a positive change of free energy (1.3 kcal/mol). However, as it was observed in the trajectory, an additional water molecule was present in

this site and establishing a hydrogen bond with the first water molecule forming the wet spot. Consideration of both water molecules in the free energy calculations revealed an energy gain of -6.6 kcal/mol, reflecting a water cooperativity effect. Another example of cooperativity effect was found in the site formed by the side-chain of Asn52 in the SH3 domain and the main-chain of Met61 in the ligand. Here, although the energy becomes more favorable by consideration of two water molecules transfer, water contribution was still not favorable. In surface sites no big negative values for free energy were found. In other calculations using the double decoupling method for free energy calculation with AMBER, the obtained values for the free energy of water in hydration sites changed from slightly positive up to -5 kcal/mol [3]. The favorable energetic impact of water molecules on complex formation was also found in a study of various protein complexes by Monte Carlo calculations using different force fields [5].

The most important conclusion that can be driven from this free energy analysis is that water molecules in wet spot sites can not be characterized uniformly in energetic terms since in some cases they manifest properties similar to cavity waters and in other do not even contribute favorably to the complex free energy (just occupying an empty space between the residues). Nevertheless, it is realistic to claim that the introduction of water into protein interface description would crucially change the energy function of the system.

4 Conclusions

We present a detailed molecular dynamics study of solvent on 17 protein complexes. Our aim has been to gain insights into the properties of interfacial solvent. We show that water molecules forming wet spots have significantly longer residence time than those on the protein surface, meaning that in terms of mobility interfacial protein residues and interfacial solvent are alike. Although interfacial water molecules are very diverse energetically, their contribution to the free energy of complex formation should be not be ignored. Our data confirm that water plays an important active role in protein interfaces, suggesting that consideration of solvent in the development of energetic functions describing protein interactions is essential.

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